CANADIAN JOURNAL OF RESEARCH

VOLUME 19 APRIL, 1941 NUMBER 4

CONTENTS

NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

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Subscription rates, postage paid to any part of the world (effective 1 April, 1939), are as follows:

	Annual	*Single Copy*
A and B	$ 2.50	$ 0.50
C and D	2.50	0.50
Four sections, complete	4.00	—

The Canadian Journal of Research is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. All correspondence should be addressed:

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. A. APRIL, 1941 NUMBER 4

THE CHINOOK WIND EAST OF THE CANADIAN ROCKIES[1]

BY H. L. OSMOND[2]

Abstract

The phenomenon known as the Chinook wind, which occurs in the foothills of the Canadian Rockies, has been investigated. A number of salient features of the Chinook have been established. The most important one is that of the pressure distribution associated with a Chinook. This involves the establishment of a pressure trough between two anticyclones, one on each side of the Rocky Mountain ridge. The location of this trough relative to the ridge is extremely important. In order that a strong Chinook occur, the trough must lie in the lee of the Rockies. It has been shown also that the region of the Chinook is in southern Alberta, extending a short distance east from the mountains.

Introduction

The Chinook wind is a meteorological phenomenon associated with the Rocky Mountains along the west coast of North America. The name itself is merely one of many local names applied throughout the world to a warm, dry wind deriving its properties from adiabatic changes. Argentina has its 'Zonda'; Sumatra its 'Bohorak'; southern California its 'Santa Ana' (4); and the Alpine countries their 'Foehns'.

The origin of the name 'Chinook' as used in North America has been accounted for by C. F. Talman in "Why the Weather". He quotes J. Neilson Barry, Secretary of the Trail Seekers' Council of Orgeon, as follows, "The name was given first of all neither to the dry wind east of the Rockies nor to the 'wet Chinook' of the coast, but to a dry northwesterly wind experienced in summer at Astoria, Oregon." This wind came from the direction of the Chinook Indian villages on the opposite shore of the Columbia River, between Point Ellice and Cape Disappointment. According to Mr. Barry the name 'Chinook' was applied as a joke about 1840 by Mr. Birnie, a Hudson's Bay factor.

The 'Three Characteristics' method of identification of Chinook winds was used by the author in his investigation. This method considers the direction of the wind, temperature changes, and the humidity of the air. As the Chinook wind is considered to result from the movement of air across or down a range of mountains (2, pp. 63-65), it always comes from a westerly direction in Alberta. To be a Chinook, a westerly wind must introduce a warmer air mass that is comparatively dry.

[1] *Manuscript received in original form October 8, 1940, and as revised, January 24, 1941.*

[2] *At the time, holder of a Bursary under the National Research Council of Canada, at the University of Toronto, Toronto, Ont. At present, Meteorologist, Stevenson Field Airport, Winnipeg, Man.*

According to Ekhart (3) this method allows too many summer occurrences of the wind to pass unnoticed. In particular, at Funsbruck in the Austrian Alps many days of Foehn occur when not all three characteristics are present. In place of the characteristics method, Ekhart adopts a slightly different set of criteria. According to him a wind from the right direction (depending on the locality) and showing a pronounced gustiness should be classified as a Chinook. However, Ekhart admits that there is the possibility of false identification by the 'gustiness' method. In the winter, there is no danger that Foehn occurrences pass unnoticed when the "three characteristics" method is used. Since only winter cases are studied here, the latter method will be used.

Results

The present writer, with the permission of Mr. J. Patterson, Controller, Meteorological Service of Canada, and under the supervision of Mr. Andrew Thomson, Assistant Controller, Meteorological Service of Canada, has investigated the occurrence during February and March, 1939, of the Chinook along the eastern foothills of the Canadian Rockies.

The following facts with respect to the Chinook wind have been established:

1. Each Chinook began after anticyclonic circulations had been established simultaneously on both sides of the mountains.

2. Before the Chinook is evident at Lethbridge and Calgary, there is a line of temperature discontinuity along the leeward side of the mountains.

3. The Chinook may begin as a gentle breeze or as a fresh wind.

4. The Chinook is generally stronger and steadier at Lethbridge than at Calgary.

5. The temperature at Calgary undergoes a greater and more sudden change at the beginning of the Chinook than that at Lethbridge.

6. The Chinook may begin at any time of day.

7. The direction from which the Chinook blows varies from the southwest through to the northwest.

8. Swift Current and Saskatoon are approximately the eastern limits to which the Chinook is still a drying wind.

9. The pressure does not increase during the Chinook period. During Chinooks lasting for days, the small diurnal pressure variation is present.

10. The advance of a Pacific air mass across the mountains into Alberta may be marked at the surface by frontal characteristics such as high humidity, low clouds, and precipitation in the north, and Chinook properties, namely, warmth and dryness, in the south. Further east, the Chinook properties diminish or disappear, and the mass may continue eastward with merely a warm front at the surface marking its progress. In these cases, a cyclonic centre develops in eastern Saskatchewan and western Manitoba.

The Typical Pressure Distribution

The first point, namely, that anticyclonic circulations must be established simultaneously on both sides of the Rockies, is most important. Math (5), in an investigation on the Chinook of Havre, Montana, stated that the pressure distribution typical for a Chinook situation was an intense low centered off the north Pacific coast and reaching inland to Alberta, with a centre of high pressure over the north Plateau* region. Ward (6, pp. 413-414), also, has

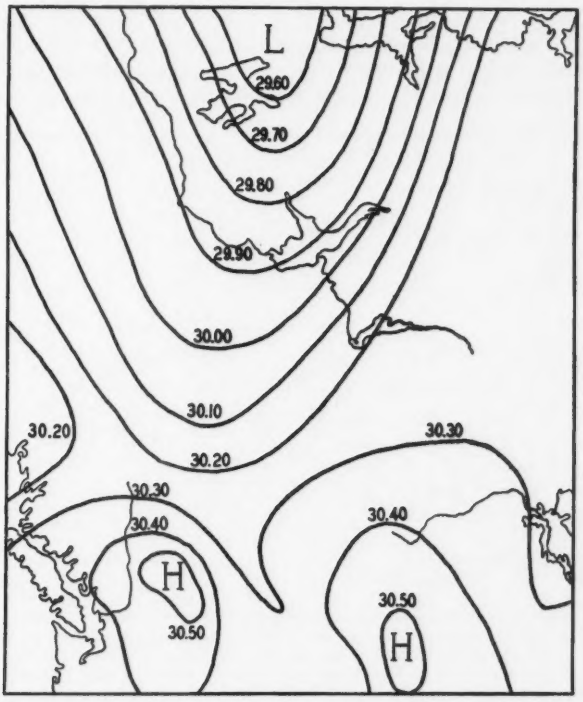

FIG. 1. *Pressure distribution (pressures in millibars) over Western Canada at 1130 hr. M.S.T., March 17, 1939. Centres of high pressure are located on both sides of the Rockies with a trough extending southward from a centre of low pressure centered in northern Canada. This is typical of the pressure distribution found to exist just before a Chinook period.*

described such a pressure distribution as being typical of a Chinook period. However, in the present investigation it was found that for each of the Chinook periods examined there were two anticyclonic centres—one on each side of the Rockies. Generally, one area of high pressure moved in off the Pacific and the other moved south from the Arctic. At the beginning of the Chinooks the Pacific high was centered over northern Oregon and southern British Columbia, near the International Boundary, while the Arctic high was

* *This is the flat plateau between the Cascades and the Rockies.*

centered just east of the foothills over the Prairies and near the boundary. Fig. 1, which shows the pressure distribution at 1130 hr. M.S.T. on March 17, 1939, illustrates conditions typical of the periods examined. With the anticyclones located in these positions, it was possible for a well defined trough to form between them on the east side of the Rockies—extending southward from the Aleutian low centered in the Bay of Alaska or from a low moving across northern Canada. On other occasions when the high pressure centres were shifted to the west, the trough formed on the windward side of the mountains, and no Chinook was observed. Also, at times the Arctic high moved southward in the neighbourhood of Hudson Bay. This gave a very broad, shallow trough extending across the prairies, with no accompanying Chinook effect, or, at most, with a very mild one. Fig. 2 shows the position

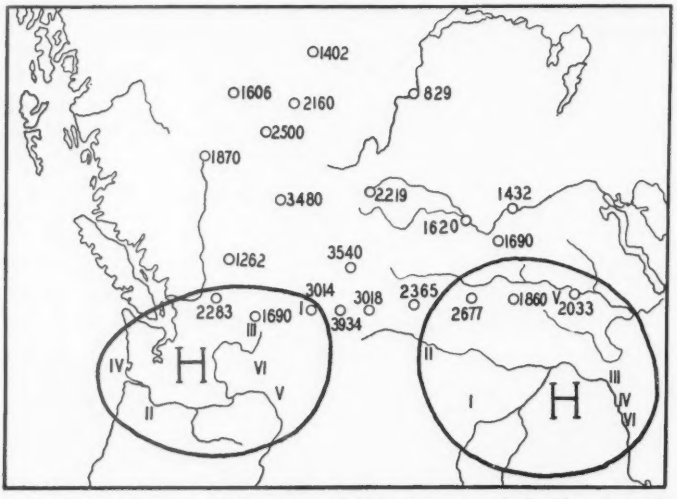

FIG. 2. *Typical locations of the centres of high pressure on both sides of the Rockies preceding a Chinook. The Arabic figures give the elevation of the terrain in feet above sea level. The Roman numerals give the location of pairs of centres of high pressure for six of the Chinook periods studied. The heavy bounding lines mark the general position of the high pressure areas—one on each side of the mountain ridge.*

of the anticyclonic centres for the six Chinook periods briefly summarized in Table I. It might be added that in checking back on pressure maps for periods discussed by previous writers (1, 5) this same distribution was found to exist.

The Line of Temperature Discontinuity Between Air Masses

The circulations about the two pressure centres result in the line of temperature discontinuity that is found to exist along the leeward side of the Rockies preceding a Chinook. The centre to the west brings in Pacific air which, although modified to a certain extent by its trajectory across land, is still moist and comparatively warm. This air mass lies on the windward side of the mountains. On the leeward side, there is the Polar Continental air

TABLE I

Date 1939	Station	Time M.S.T.	Wind From	Wind To	Temperature change	Humidity* change	Sky** condition	Eastern limits of the chinook effect***	Pressure tendency	Pressure distribution	Following weather
Feb. 10	Lethbridge	12 hr.	S5	SW6 Vel. increased to 55 m.p.h. by 17 hr.	18° rise in 2 hours	R.H.—15%	Clr.	Medicine Hat	Rate of decrease accelerated	Centres of high pressure at Cranbrook, B.C., and northeast of Billings, Montana. Both moved southward rapidly	Polar front passed late on the 11th bringing light snow and fog until the middle of Feb. 12
	Calgary	15 hr.	S5	SW10	10° rise in few minutes	Dropped sharply	Clr.		Falling steadily before and after		
Feb. 16	Lethbridge	01 hr.	S16	SW10 Vel. increased steadily	Diurnal decrease checked. At 03 hr. rose 10° in 1 hour	R.H.—20%	Ovc. Ac.	Medicine Hat	Falling steadily before and after	High pressure centres at Portland, Oregon, and Havre, Montana on the 2330 hr. chart of Feb. 15. Western high continued southward; coastal centre did not move until Feb. 17	Polar front moved slowly southward during the 17th and 18th. Snow showers reducing visibility and ceiling until the afternoon of Feb. 21
	Calgary	01 hr.	SW5	W8 Vel. 19 m.p.h. next hr.	9° rise in 1 hour	R.H.—30%	Ovc. Ac.				
Feb. 21	Lethbridge	20 hr.	SE3	SW9	4° rise in 1 hour	R.H.—30% in 2 hours	Hi. sctd.	Medicine Hat	Falling steadily before—steady for 7 hours, then falling again	High pressure centres at Grand Forks, B.C. and north of Bismarck, N.D. The N.D. high moved south rapidly, but a high was maintained west of the Rockies	Polar front followed by light snow and fog which limited visibility and ceiling during Feb. 23 and morning of Feb. 24
	Calgary	23 hr.	NW1	W2	22° rise in 2 hours	R.H.—18%	Hi. sctd.		Falling steadily before and after		
Feb 24	Lethbridge	0830	S17	SW8	5° rise in 1 hour	R.H.—9% *w*, 0.1 gm.	Hi. sctd.	Medicine Hat	Began to fall before the Chinook and continued until the wind shifted to the northwest at both stations	Anticyclones centred at Bismarck, N.D., and off the mid-Pacific coast	Polar air advanced. Snow reduced ceiling and visibility on Feb. 25
	Calgary	1930	SW4	W11	8° rise in 1 hour	R.H.—25%	Hi. sctd.				
Mar. 10	Lethbridge	02 hr.	NE6	W10	No diurnal decrease	R.H.—10%	Clr.	Medicine Hat	Slow decline until 10 hr. Rapid fall next 6 hours, then steady	Centres of high pressure southeast of Spokane and east of Regina. Spokane centre moved south and Regina centre moved east at about the same time	Occluded system moved in off the Pacific behind the Chinook. Then the Polar front moved south bringing light snow and fog during March 12 and 13
	Calgary	1830	W2	W14	6° rise in 1 hour	R.H.—20%	Clr.		Falling steadily until 19 hr. then steady for 7 hours before falling again		
Mar. 17	Lethbridge	1125	S4	SW7	7° rise in 1 hour	R.H.—15% *w*, 0.2 gm.	Hi. sctd.	Swift Current	Falling steadily before and after	High centres at Spokane and Bismarck at 1730 hr. on March 17. Bismarck high moved southeast; Spokane high remained until March 24	Warm Chinook continued until March 24, then the Polar air advanced
	Calgary	19 hr.	S6	W21	12° rise in 1 hour	R.H.—15% *w*, 0.6 gm.	Hi. sctd.		Falling rapidly except for steady period from 19 to 21 hr.		

** The change in moisture content of the air at the station is given in terms of the relative humidity (ratio of actual vapour pressure to the saturation value as a percentage). The values of 'w' when given indicate the grams of water per kilogram of air. **Abbreviations used: clr.—clear; ovc.—overcast; ac.—altocumulus clouds; hi.—high; sctd.—scattered. ***The air mass frequently moves farther east but loses the fundamental Chinook property—dryness.*

brought in by the Arctic high. The P_C air remains but slightly modified, as the terrain over which it has been brought is very similar to that found in its source region during the winter months. As a result, the air mass on the leeward side of the mountains is stable, dry, and cool. Thus, the originally homogeneous air mass is modified in an entirely different way on either side of the mountains—with the ridge of land acting, as it were, like a bulwark separating them.

The establishment of the well defined trough between the anticyclones finally breaks down the imaginary wall separating the two masses. It was observed that, while the trough existed, the Pacific high remained stationary, whereas the Arctic centre moved southeastward. At the same time the Chinook made its appearance.

The Direction from which the Chinook Blows

There seems to be very little regularity in the Chinook winds—except in direction. In the periods examined they were evident at Lethbridge earlier than at Calgary. The wind began as a gentle breeze or as a fresh wind, sometimes attaining velocities of 40 to 50 m.p.h. At Lethbridge, the records show that the Chinook blows from the southwest or west and is strikingly persistent for its duration. The most marked example of this was the period March 18 to 24, 1939, when the wind blew constantly from the southwest or west (with the exception of 10 hr. when it was temporarily halted) with an average velocity of 26.3 m.p.h. At Calgary the Chinook wind comes from the west but is not nearly as steady as at Lethbridge. The velocity is generally somewhat less at Calgary. These consistencies and inconsistencies are related to the topographical locations of these two stations. Lethbridge is situated at the mouth of a valley running in an east-west direction, whereas Calgary is exposed on an open slope. The duration of the Chinook varies from a few hours to a few days, as shown in Table II.

TABLE II

DURATION (IN HOURS) OF CHINOOK WINDS BEGINNING ON THE GIVEN DATES

	February				March		
	10	16	21	24	10	17	19
Duration at Lethbridge	29	61	25	20	36	35	142
Calgary	21	57	3	4	13	21	

It has been mentioned that from March 19 to 24 Lethbridge experienced very consistent westerly winds. Although the temperature, humidity, and pressure curves for Calgary are very similar to those given for Lethbridge for the period March 18 to 24, the winds at Calgary were not nearly as consistent as those at Lethbridge. The winds at Calgary had a velocity generally less

than 10 m.p.h.; they came from all directions but were predominantly westerly. Table III gives the number of miles of wind from the various directions recorded at Calgary from March 20 to 23, inclusive.

TABLE III

MILES OF WIND RECORDED AT CALGARY, MARCH 20 TO 23, 1939

Blowing from	N	E	SE	S	SW	W	NW
Miles of wind	45	3	47	83	114	193	84

Variability of the Chinook

The Chinook wind may begin at any time of the day. Table I shows that, in these few examples, at Lethbridge the first effect of the Chinook was felt at noon, in the evening, at midnight, and in the morning. The same is true for Calgary, but here the first effects are felt generally later than at Lethbridge. Frequently, it was noticed that when Lethbridge was experiencing the Chinook, at Calgary the temperature rose and the humidity declined with a south wind. The changes at Calgary were due to the "side-tracking" of the Chinook air from the vicinity of Lethbridge by the prevailing anticyclonic circulation. When the wind at Calgary veered to the west and the real Chinook began to blow, the changes in temperature and humidity were accentuated.

The temperature change accompanying a Chinook is generally more marked at Calgary than at Lethbridge. When the wind began blowing at night it was observed that at Lethbridge merely the normal diurnal cooling was checked while there was a definite warming at Calgary. When the Chinook makes its initial appearance during the day, there is at Lethbridge an accelerated temperature increase but at Calgary there is a definite jump in the temperature. A good example is that of March 17, 1939, when the Chinook began in the heat of the day at 1125 hr. at Lethbridge, accompanied by a temperature increase of 7° F. within an hour; while at Calgary it began at the beginning of the cooling period—at 1900 hr.—and was marked by a 12° F. rise within the next hour.

Air Mass Changes at Edmonton

Red Deer and Edmonton, which are 80 and 170 miles respectively north of Calgary, seldom experience the true Chinook wind. When the southern stations are under the influence of the drying wind, the northern stations often experience a westerly wind that is warm but moist. This difference in the air properties is probably related to the fact that the mountains recede to the northwest, leaving a wide flat region between them and the northern stations. Also the mountains are considerably lower in the northern section. However, there are times when a west wind at Edmonton is a Chinook, exhibiting the property of relative dryness. These winds are generally of shorter duration than those in the southern part of Alberta. Usually, a Chinook in the southern section is accompanied by an advance of warm

moist air from the Pacific to Edmonton, provided that the Polar front lies to the north of it. It was seldom that upper air data were available for the Alberta region. However, data taken by observers in airplanes were obtained from March 17 to 22, 1939. The graphs in Fig. 3 show that between March 17 and 18 the structure of the air above Edmonton was altered completely. On March 17, there was a surface inversion of 16° C. up to the 0.55 km. level. Above that, the air column was isothermal up to 3.9 km. at least. On March 18, the air was much warmer and the lapse rate in the layer from 0.9 km. up to 3.2 km. was about 2.5° C. per 1000 ft. There was an inversion of 11.5° C. up to the 0.9 km. level. The mixing ratio had increased five- to ninefold and the relative humidity was much higher except for the surface levels. It is quite evident that between 0647 hr. on March 17 and 0648 hr. on March 18, moist Pacific air replaced the cooler and drier Polar air.

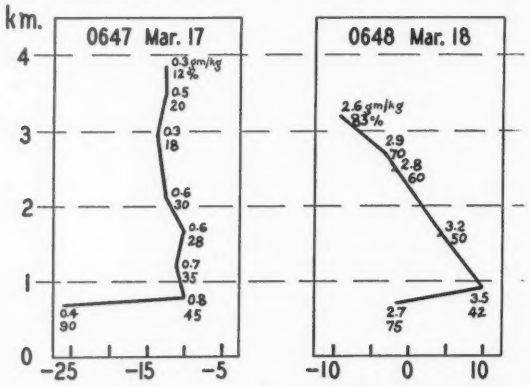

FIG. 3. *Ascent graphs for Edmonton, showing the moisture and temperature variations with height at the same time for two consecutive days, March 17 and 18, 1939. Temperature in degrees centigrade is plotted against height in kilometres. The moisture content at different levels is expressed in two forms—specific humidity (grams of water per kilogram of air) and relative humidity (ratio of actual vapour pressure to the saturation value as a percentage). The graphs show that the air mass over Edmonton was much warmer and more moist on the second day. At the same time a strong Chinook was blowing in southern Alberta.*

Change in Humidity as the Chinook Air Moves Eastward

The passage eastward of the Chinook air is accompanied by a rapid change in its relative humidity. The air has an increased relative humidity at Medicine Hat which is only 100 miles east of Lethbridge. By the time it reaches Regina the air can no longer be said to be dry. In the periods examined it was found that the specific humidity of the surface layer did not change noticeably as the air moved east. Thus the increase in the relative humidity of the lower level may be due to the decrease in temperature resulting from the cooling effect of the surface traversed, rather than to the picking up of moisture. On the other hand, the upper levels of the air mass may have become more moist by the rising of moisture from below. Then the heat of evaporation required to maintain the moisture content at the

surface would account for decreasing temperatures, and, thus, increasing relative humidity. Closely related to this problem is the question whether the Chinook phenomenon is merely a surface effect or one involving a considerable thickness of the atmosphere. If only the surface layer is involved, there would be a temperature inversion in the lower levels. This, in turn, would eliminate the possibility of the rise of moisture to the higher levels On the other hand, if the whole air mass is involved there would be a lapse rate approaching the dry adiabatic—on the assumption that the air has moved down the mountain slope. Such a condition might conceivably favour the rise of moisture. The winds aloft in southern Alberta which were observed to be from the west and of increased intensity during a Chinook, and the complete air mass change at Edmonton as shown by Fig. 3, would seem to favour the second possibility. This can be checked only when additional upper air data are available. A further factor indicative of such a process is the gradual increase of cloudiness from west to east during a Chinook period.

The Appearance of Warm Frontal Characteristics

Under certain circumstances the original Chinook air continues eastward as a warm front at the surface. This was found to be so when cyclonic centres originating in the Aleutian low advanced east along the boundary of the Northwest Territories to Saskatchewan and then southeast to the Great Lakes. A series of cyclonic centres formed and moved across northern Canada during the period March 18 to 24. These systems developed in the trough, which extended in a southeasterly direction from the low centered in the Gulf of Alaska. The first centre to form in the trough was observed on the 0530 hr. chart of March 18, and was located near McMurray (240 miles north of Edmonton), where the pressure was reported to be 29.54 in. at that time. The low advanced southeast from McMurray, reaching Winnipeg by the time of the 2330 hr. chart on the same day. At this point the warm air, which was the original dry Chinook air in the foothills of Alberta, took on distinctive frontal properties. Precipitation was reported at Winnipeg at 1730 hr. on March 18, and preceded the front as it advanced eastward across the Great Lakes and out onto the Atlantic.

The Advance of the Polar Front

From March 20 to 24, four centres of low pressure that followed the McMurray low moved eastward just along the 55th parallel until finally there was a broad band of low pressure extending from the Alaskan low across the northern part of the prairies and along the west shore of Hudson Bay. When the high pressure west of the Rockies began to weaken, the polar front lying in the band of low pressure advanced south, finally passing Lethbridge on March 25.

The termination of a Chinook is generally brought about by a wind shift to a northerly direction, marking the advance of the Polar Continental air. Math (5) spoke of the battle between the Chinook and the P_C air masses.

In the March 18 to 24 period it was noted that the Chinook was temporarily halted on March 19. This coincides with the southeastward movement of the low from near McMurray to Winnipeg. The cyclonic circulation about this centre brought fresh P_c air from the north into the Chinook region. But the plateau high remained in position. Thus, with the development of another high just east of the Rockies, resulting in a new trough formation, the Chinook was restored. Later, on March 24, the band of low pressure advanced southward, bringing in polar air behind it. This time the plateau high dissipated and the Chinook period was brought to a close.

The warm sunny period of the Chinook is followed by unsettled weather, bringing cloudy skies, chilly winds, snow storms, and generally foggy conditions in the foothills. On rare occasions an occluded front moves in from the Pacific with the withdrawal of the western high. The advance of the P_c air is then delayed until the passage of this front.

Conclusion

From the results of this investigation the writer believes that a Chinook may be safely expected in the region from Lethbridge north to Calgary when anticyclonic circulations are established on both sides of the Rockies so as to allow the formation of a trough in the lee of the mountains. Warm, moist air may at the same time be brought across the mountains further north. The distance that this air mass moves east will depend on its own energy and the strength of the cooler air being displaced. The drying property of the Chinook air soon disappears as it moves east. If a cyclonic centre develops in the north and moves southeast, a warm front will develop and move across the Great Lakes region. The Chinook is generally terminated by the advance of polar air following the removal of the high pressure areas.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. B. APRIL, 1941 NUMBER 4

A TRYPSIN-INHIBITING FRACTION OF ASCARIS[1]

BY H. BRUCE COLLIER[2]

Abstract

A crude extract of *Ascaris lumbricoides* strongly inhibits pepsin and trypsin but not papain; the extract shows no proteolytic activity. An active trypsin inhibitor was isolated by fractional precipitation. It is not precipitated by trichloracetic acid and has the properties of a polypeptide.

The inhibitor acts instantaneously on trypsin, exerting its maximum effect at neutral and acid reactions, with a minimum at pH 5. The effect of concentration suggests a reversible combination between enzyme and inhibitor. The inhibitor has no effect on papain, but stimulates peptic digestion.

Introduction

Weinland (18) in 1903 suggested that intestinal helminths produce anti-enzymes that protect them from attack by the digestive enzymes of the host. (Although the term 'anti-enzyme' has been current, it seems preferable to follow Northrop's usage and employ 'enzyme inhibitor' in order to avoid confusion with immunological phenomena.) Mendel and Blood (10) a few years later showed that extracts of Ascaris inhibited pepsin and trypsin but not papain.

The present investigation records an attempt to isolate the enzyme inhibitor of Ascaris. A trypsin-inhibiting fraction was obtained and partially purified; the very small yield made further purification impracticable. However, in view of the fact that the investigation is not being pursued further at the present time, it seemed worth while to record the methods employed and the general results obtained.

Sang (14) has recently prepared from Ascaris an extract that showed both inhibitory and proteolytic activity. Since he measured digestion by means of the formol titration, which is not specific for proteolysis, it is probable that the results were complicated by the presence of peptidases. Von Bonsdorff (4) prepared an extract that showed proteolytic activity but no inhibitory effect on pepsin, trypsin, or papain.

Trypsin inhibitors have been isolated from other sources: from egg-white by Balls and Swenson (2); from pancreas by Kunitz and Northrop (8); and from blood plasma by Schmitz (15). The preparation of a crystalline, specific inhibitor from pancreas removes any doubt that such 'anti-enzymes'

[1] *Manuscript received in original form, November 13, 1940, and as revised, January 3, 1941. Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.*

[2] *Lecturer.*

actually exist. It is interesting that these inhibitors from various sources all have somewhat similar properties.

Experimental

Methods

Crystalline pepsin was prepared from Merck's U.S.P. Pepsin by the method of Philpot (12). Commercial papain was partially purified by thrice repeated precipitation from aqueous solution by ethanol at a concentration of 55% by volume. Trypsin was prepared from active pancreatic extracts by the method of Northrop (11, p. 141). It failed to crystallize but had a specific activity, $[T.U.]_{mg.N.} = 0.07$, approaching that of crystalline trypsin.

Proteinase activity was measured by the elegant method of Anson (1), using a dialyzed solution of "Difco" Bacto-Haemoglobin as substrate, the concentration of the stock solutions being determined by iron estimations. This preparation was found to give a very low blank—only 0.0001 m.e. of tyrosine per 5 ml. of filtrate. The digestions were carried out for 10 min. at 37° C. but temperature corrections have been applied and the results converted to Anson units. The samples may be either centrifuged or filtered, after precipitation with trichloracetic acid. Filtration was found to give slightly higher values, using Whatman No. 2 paper, which contains a trace of chromogenic material extractable by the acid. ($N/5$ trichloracetic acid passed through the paper gave a colour value equal to 0.000016 m.e. of tyrosine per 5 ml.)

The colorimetric measurements were made in a Klett-Summerson photoelectric colorimeter, a Corning red filter No. 241 being used. The volumes employed were smaller than those recommended by Anson. Into a colorimeter tube were pipetted 1.5 ml. of trichloracetic acid filtrate, 3.0 ml. of 0.5 N sodium hydroxide, and 1.0 ml. of the Folin-Ciocalteu reagent, diluted three times. The method was standardized against pure tyrosine, estimated under identical conditions. Up to a tyrosine concentration of 0.0016 m.e. per 5 ml. there was a virtually linear relation between concentration and colorimeter reading, against a reagent blank.

Enzyme or buffer solutions must not be preserved with thymol, which reduces the reagent. Purified enzyme and inhibitor solutions give a virtually negligible blank, but with crude extracts the method of performing control determinations is of great importance. The blank values for substrate, enzyme, and inhibitor, determined separately, are greater than when enzyme and inhibitor are mixed, and substrate added, followed immediately by trichloracetic acid. The latter procedure was always adopted. Boiled enzyme controls also deviated considerably from the above. In each case the values for the appropriate blanks have been deducted from the published results, or more frequently, the blank tube was used in adjusting the colorimeter to zero reading.

Activity of Crude Ascaris Extracts

Live specimens of *Ascaris lumbricoides* were obtained from hogs (through the kind co-operation of Dr. E. Dufresne, Inspector-in-Charge, Wilsil, Ltd., Montreal), and were kept in normal saline at 37° C. until used. Preliminary tests were made for proteinase activity. The worms were dissected and body wall, ovaries, intestine, and oesophagus separately ground with sand and extracted with saline, the filtered extracts being tested for pepsin, trypsin, cathepsin, and papain. The ovary and gut showed traces of cathepsin activity, and the gut, traces of trypsin; the other tests were negative. In any case, the observed activities were not sufficient to interfere with the inhibition tests. In view of the proteolytic activity frequently reported by other workers, it was thought that the worms might contain a proteinase capable of digesting albumin or casein, but not haemoglobin. However, extracts of whole Ascaris had no effect on egg albumin at pH 5 or on casein at pH 6, using the colorimetric method. Nor did a concentrated inhibitor preparation digest casein at pH 4, as measured by 'tyrosine' and nitrogen determinations on the trichloracetic acid filtrate.

For tests of inhibitor activity, body walls of fresh worms were dissected out, washed in cold running water, ground with sand and extracted with 1% sodium chloride (100 ml. per 25 gm.) overnight in the presence of toluene. The extract was filtered, shaken with ether, separated and dialyzed. This yielded a pink, opalescent liquid containing much glycogen. Preliminary tests indicated that this extract strongly inhibited pepsin and trypsin, but had no effect on papain.

In the case of crystalline pepsin, 0.05 ml. of 0.005% solution was treated with Ascaris extract, in a total volume of 1.0 ml. at pH 2, for 15 min. at room temperature, then was allowed to act on haemoglobin. The results were as follows:—

Pepsin alone	1.15×10^{-4} P.U.	
plus 0·2 ml. extract	1·07	= 7% inhibition
0.6	0.36	= 69
1.0	0	= 100

One millilitre of extract, at pH 7, was added to 0.10 ml. of 0.01% trypsin, which after 15 min. at room temperature was allowed to act upon substrate:

Trypsin alone	2.35×10^{-4} T.U.	
plus extract	1.20	= 49% inhibition

Using 0.10 ml. of 0.125% papain, activated with potassium cyanide, and 1 ml. of extract, the following results were obtained:—

Papain alone	5.1×10^{-4} m.e. per ml.
plus extract	5.0

Purification of Inhibitor

Preliminary tests showed that the inhibitor in the crude extract was not affected by boiling nor by 2.5% trichloracetic acid. It was precipitated by 85% ethanol and by 0.7 saturated ammonium sulphate. As the inhibitor

seemed similar to that of Northrop (11, p. 135), his procedure was applied, with certain modifications.

Whole specimens of Ascaris were minced and extracted with 0.25 *N* sulphuric acid. Addition of ammonium sulphate to 0.3 saturation resulted in the precipitation of much inert protein, together with the pepsin inhibitor and some trypsin inhibitor. Addition of ammonium sulphate to 0.7 saturation gave a precipitate containing trypsin inhibitor and a large quantity of glycogen. Attempts to recover the pepsin inhibitor by reprecipitation resulted in complete loss of activity, and this phase of the investigation has not been pursued further.

For direct isolation of trypsin inhibitor the method has been simplified as follows. Several methods of mincing the worms were tried, the use of the Waring Blendor* having been found most convenient. The worms, being placed in 1% saline in the Blendor, are reduced in a few minutes to a fine suspension. This was autolyzed under toluene for several days, in the presence of a little diastase to insure removal of glycogen. Trichloracetic acid was then added to 2.5% concentration and the fluid was heated to 80 °C. for five minutes, then cooled and filtered. Treatment with charcoal removed the colour and the very disagreeable odour but did not affect the inhibitor. This extract was adjusted to pH 3.0 by addition of 5 *N* sodium hydroxide and was saturated with magnesium sulphate, which precipitated the inhibitor. The precipitate was filtered off by suction through hardened paper and was found to be a very active inhibitor. Since treatment of several kilograms of worms yielded only 300 mg. of moist precipitate at this stage, the purification was not carried further. The precipitate was dissolved in 3 ml. of water, giving a stock solution that contained 7.0 mg. of total nitrogen per ml. This was diluted tenfold for the following experiments.

Properties of Inhibitor

The inhibitor was not affected by boiling nor precipitated by 2.5% trichloracetic acid. It was precipitated by saturated magnesium sulphate and by 0.7 saturated ammonium sulphate. It gave a pink biuret test, positive Sakaguchi test for arginine, positive Millon's test, negative Molisch test, negative nitroprusside test for free or oxidized sulphydryl groups, negative Sullivan test (17) for guanidine, negative Lison's toluidine blue test (9) for sulphuric acid esters, negative lead acetate test for unoxidized sulphur.

Ferrocyanic, picric, and trichloracetic acids failed to precipitate the inhibitor. However, phosphotungstic acid gave a slight precipitate, and tannic acid a heavy one. Copper sulphate and mercuric chloride gave precipitates in slightly alkaline solution. The inhibitor has the properties of a proteose or polypeptide; it dialyzed only very slowly through cellophane.

* *Waring Corporation, 1697 Broadway, New York City. The Blendor is very useful for mincing and extracting animal tissues, suspended in a fluid medium.*

Effect on Papain

One millilitre of 0.025% papain, activated with potassium cyanide, was treated with 0.20 ml. of 1/10 inhibitor for 15 min. at room temperature, and the activity determined in the usual manner. No inhibition was apparent.

Papain alone	1.71×10^{-4} m.e. per 5 ml.
plus inhibitor	1.74

Effect on Pepsin

One millilitre of 0.001% pepsin was treated with 0.20 ml. of 1/10 inhibitor for 15 min. at room temperature, and the activity was determined. In one pair of tubes the pepsin was dissolved in 0.1 *N* hydrochloric acid; in another, in 0.1 *M* acetate buffer, pH 5.5. The results showed a strong stimulation of pepsin digestion by the inhibitor. This was not due to protection from destruction at the higher pH, as the results indicate. Nor was it due to action of pepsin on the inhibitor itself, as controls showed no increase in the blank values.

pH 1.1	Pepsin alone	0.75×10^{-4} P.U.
	plus inhibitor	1.30
pH 5.5	Pepsin alone	0.68
	plus inhibitor	0.90

Effect on Trypsin

The effect of time on trypsin inhibition was investigated as follows. To 1.00 ml. of 0.003% trypsin was added 0.05 ml. of 1/10 inhibitor, both being at pH 7. After a predetermined time at room temperature, substrate was added, and the activity determined. The results (Table I) indicate that maximum inhibition occurred at zero time, and that a prolonged interval slightly decreased the effect. The blank did not change with time.

TABLE I

EFFECT OF TIME INTERVAL ON TRYPSIN INHIBITION

	Activity, T.U. $\times 10^4$	Inhibition, %
Trypsin alone	3.32	0
Trypsin plus inhibitor, 0 min.	1.05	68
Trypsin plus inhibitor, 10 min.	1.55	53
Trypsin plus inhibitor, 20 min.	1.57	53
Trypsin plus inhibitor, 30 min.	1.57	53

The effect of inhibitor concentration is illustrated in Table II. In each case, inhibitor was added to 0.10 ml. of 0.03% trypsin and the volume made up to 1.00 ml. After 10 min. at room temperature, the resultant activity was determined. The degree of inhibition does not vary directly as inhibitor concentration; this suggests an equilibrium between free trypsin, inhibitor, and the inhibitor-enzyme complex.

In investigating the effect of pH on inhibition, 0.10 ml. of 0.03% trypsin and 0.05 ml. of 1/10 inhibitor were added to 1 ml. of 0.1 *M* phosphate buffer

TABLE II

EFFECT OF CONCENTRATION ON TRYPSIN INHIBITION

	Activity, T.U. × 10^4	Inhibition, %
Trypsin	3.50	0
Trypsin plus 0.02 ml. inhibitor	2.60	26
Trypsin plus 0.05 ml. inhibitor	1.50	57
Trypsin plus 0.10 ml. inhibitor	0.70	80
Trypsin plus 0.20 ml. inhibitor	0.10	98

of the desired pH. After 10 min. at room temperature, the activity was determined. In each case, parallel controls without inhibitor were run to determine the effect of the reaction on the enzyme alone. In the tests at pH 1 and pH 3, immediately after addition of substrate sufficient alkali was added to neutralize the buffer. If the buffer were neutralized *before* the addition of substrate, the observed inhibition was somewhat less. The results (Table III) indicate that minimum inhibition occurred at pH 5. There was no evidence of a reversal of inhibition in strongly acid reaction, such as was observed by Northrop in the case of pancreas inhibitor.

TABLE III

EFFECT OF pH ON TRYPSIN INHIBITION

pH		Activity, T.U. × 10^4	Inhibition, %
1	Trypsin	3.17	
	Trypsin plus inhibitor	1.03	67
3	Trypsin	3.20	
	Trypsin plus inhibitor	1.50	53
5	Trypsin	3.23	
	Trypsin plus inhibitor	1.76	45
7	Trypsin	3.20	
	Trypsin plus inhibitor	0.84	74

Enzyme-inhibitor Interaction

The reactive 'tyrosine' and acid- and alkali-titratable groups of enzyme and inhibitor before and after mixing were determined, in the hope that any observed changes might throw light on the nature of the active groups involved. The observed changes were small, and since the inhibitor was not pure, probably are of little significance. There was found to be a small decrease in the acid- and alkali-titratable groups, and a very small increase in reactive 'tyrosine'. Exactly neutral solutions of enzyme and inhibitor showed no pH change on mixing.

In the course of these experiments it was observed that trypsin, treated with inhibitor, was no longer precipitable by trichloracetic acid, even on boiling. The precipitate given by phosphotungstic acid was appreciably less than that given by the separate components, whereas that given by tannic acid was not noticeably altered, nor was the precipitation by heavy metals affected. The complex resembles pepsin in not being precipitable by trichloracetic acid (2.5%), but showed no peptic activity. A solution of crystalline pepsin was no longer coagulated by boiling, after the addition of trypsin inhibitor.

Discussion

It has been demonstrated that crude extracts of Ascaris strongly inhibit pepsin and trypsin but have no effect on papain, thus confirming the results of previous workers. The extracts were devoid of proteinase activity. Ascaris probably cannot assimilate undigested protein, but it is surprising that cathepsin was not found in more than traces, for protein synthesis certainly takes place. Demonstration of strong proteinase activity by other workers may have been due to bacterial contamination or to the use of non-specific methods of estimation.

The trypsin inhibitor isolated from Ascaris has the properties of a proteose or polypeptide and is similar to the inhibitors that have been obtained from egg-white, pancreas, and blood plasma. Its function presumably is to protect the parasite from attack by the proteinases of the digestive tract of the host: there is no inhibitor for the papain-like enzymes, which are able to destroy live worms. This has been demonstrated by Robbins (13) with ficin, and with papain and bromelin by Berger and Asenjo (3). On the other hand, Burge and Burge (5) have found that *dead* parasites are digested by trypsin, so that the presence of inhibitor cannot be the only protective factor. Northrop (11, p. 14) has discussed the resistance of living tissue to digestion, and has concluded that permeability is an important factor.

The occurrence of inhibitors in various sources associated with the presence of trypsin suggests that this may be a general biological phenomenon; that is, the inhibitor may appear whenever living tissue is in contact with active enzyme. If the inhibitor is actually functional, and not accidental, and if the intestinal helminths have evolved from free-living forms, it is highly improbable that this mechanism has arisen *de novo*, but more likely that it is an adaptation of an already existent physiological mechanism.

It has been assumed that the inhibitor can diffuse from the worm into the gut of the host. Stewart (16) concluded that protein digestion by the host is thus inhibited, but gave no evidence that the inhibitor is actually excreted by the parasite. Sang (14) found that a living worm inhibited a tryptic digest of casein, but he did not eliminate the possibility of pH change due to production of organic acids. The author observed that living Ascaris imparted an antitryptic effect to saline, tested after neutralization, but it is not known whether the specific inhibitor was responsible.

The mode of action of the trypsin inhibitor is a problem of great theoretical interest, but the examination of a relatively impure preparation cannot shed much light on the actual mechanism of inhibition. However, it seems worth recording that when enzyme and inhibitor combine there is a decrease in acid- and alkali-titratable groups, and a small increase in reactive 'tyrosine'. This may possibly be related to the increase in 'tyrosine' during protein digestion.

The properties of the inhibitor are somewhat different from those observed by Northrop with his pancreatic preparation, in that minimum inhibition takes place at pH 5, being greater at pH 1 and pH 7; further, the reaction appears to be instantaneous. Horwitt (7) has pointed out that trypsin is inhibited by acid substances, as heparin, and by certain basic dyes; it is possible that the Ascaris inhibitor may be either an acid or a basic peptide. (Herriott's (6) pepsin inhibitor appears to be a strongly basic peptide.) Since the trypsin-inhibitor complex resembles pepsin in its precipitation properties, the complex may be a more acid protein than trypsin itself, and the inhibitor an acid peptide.

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FACTORS AFFECTING THE PETT VISUAL TEST FOR VITAMIN A DEFICIENCY[1]

By L. B. Pett[2] and Marian K. Lipkind[3]

Abstract

Under the conditions prescribed for the Pett test, which is described, it has been found that: (i) antecedent light does not affect the median of the three tests usually performed; (ii) no error results from the wearing or not wearing of glasses; (ii) the use of pilocarpine to contract the pupils is not advisable; (iv) some light may be admitted into the test room; (v) the time of exposure to the bright light (30 sec.) is suitable; (vi) repeated tests cause a 'learning' effect so rarely as to be of little concern; (vii) the standard error of the mean is ± 1.4 and of the median is ± 2.4 sec. Some results are presented suggesting that a diurnal rhythm in the vitamin A content of the blood exists.

Introduction

The last ten years of vitamin research have suggested the importance of accurate tests for vitamin deficiencies in human beings, in order to facilitate the application of results obtained in animal experimentation. Such tests are also important because recent research tends to show that slight deficiencies of vitamins, often called 'subclinical', may be the causes of various vague ills of mankind. This paper reviews some details of a rapid visual test for vitamin A deficiency, and presents new information on factors that may affect it. The paper might be considered No. 4 of a series.

Many factors affect visual tests for vitamin A deficiency. The importance of studying these factors has been emphasized in recent papers by Harris and Abbasy (2, 3), and by Thomson *et al.* (8, 9). The latter give detailed accounts of investigations with a somewhat limited number of subjects, using certain methods, followed by sweeping generalizations. Of all the methods for detecting vitamin A deficiency, the Pett test is the simplest and most rapid, and is the only test correlated with blood analyses. Since it was not included in the study made by Thomson *et al.* (8, 9), a similar but more extensive study of influencing factors is here reported.

Numerous tests for night blindness, dark adaptation, etc., have been described and are reviewed by Pett (6). Several of these tests have been studied in regard to influencing factors, and several seem to fail in the most important factor, one that should influence all tests purporting to measure vitamin A deficiency, namely, the actual relationship of vitamin A therapy to the test.

[1] *Manuscript received in original form September 30, 1940, and as revised, January 13, 1941. Contribution from the Department of Biochemistry of the University of Alberta, Edmonton, Alta., assisted from a grant to Prof. G. Hunter from the Associate Committee on Medical Research of the National Research Council. Part of this work was included in a thesis for the degree of Master of Science in the University of Alberta.*

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It has been shown (5, 6, 7) that the testing of hundreds of people for vitamin A deficiency permits construction of a frequency distribution graph. The importance of such a graph was first emphasized by Pett (6). Part of this graph represents people deficient in vitamin A, and the dividing line can be decided only by selecting some as "deficient", administering vitamin A, and finding whether the test shows a difference or change toward the "normal" end of the curve. Pett (6) reported 200 serial cases classified as "deficient", who responded to vitamin A therapy. Since that publication many hundreds more have shown the same result, thus proving that the Pett instrument (now called the Vitometer) responds to vitamin A therapy of "deficient" individuals. Correlation with actual blood analyses for vitamin A has also been reported (7). These two important factors are frequently lost sight of in the discussions of other instruments and the accuracy with which they measure 'subnormal dark adaptation', etc.

The following factors are discussed in the present paper: the effects (i) of antecedent light, (ii) of wearing or not wearing glasses, (iii) of contracting the pupil with pilocarpine, (iv) of the lightness of the test room, (v) of the duration of exposure to bright light, (vi) of repeated tests in causing "learning", and (vii) the variability to be expected in different tests on one person. In addition some results are presented suggesting the possibility of a diurnal rhythm in the vitamin A content of the blood.

Experimental Procedure

It is supposed that the recovery of vision after blinding by a bright light depends on a re-formation of the retinal visual purple from vitamin A. Since 1865 many means have been used for studying this general process, and many of them are very complicated. Since the relationship to vitamin A became clearer, numerous tests have been suggested. The conditions embodied in the Vitometer were arranged after many variations had been tried by actual experimentation on many subjects. The resulting test has a simplicity and rapidity of definite advantage, and an accuracy sufficient for most purposes. The following extracts from Pett (6) will serve to describe the apparatus:

"*Description of Apparatus*—The instrument here outlined consists of a black metal (or wooden) box, about 1 cubic foot in volume. Protruding from the front is a headpiece, and on the back are various instruments. Inside the box are: (1) a source of bright white light for bleaching visual purple in the eyes, coming from either one or several bulbs. (2) A surface for reflecting this bright light into the eyes, while looking into the headpiece, such that 50 foot-candles is the intensity at the eyes; the bright light is connected to a voltmeter and rheostat, and each instrument is calibrated by a photometer to operate at a certain voltage; the reflecting surface is paper (English Cartridge Paper, 100 pounds), is 12 inches square, and covers a metal shield from which two rectangles are cut out. (3) Behind the metal shield is a dim light, so placed as to shine evenly through the two rectangles. In passing through the paper this light loses 90 per cent of its intensity and at

the eye has an intensity of about 4 millifoot-candles, and is slightly reddish. A mechanical device controlled from the back of the box is used to cover one of the rectangles of dim light, while the other remains visible, the subject then having to distinguish between vertical and horizontal directions.

"*Description of a Test*—The subject adjusts his head comfortably against the rubber forehead rest, wearing glasses if they are usually worn, and is shown the rectangles of dim light, one "Up", one "Across". The bright light is put on, and he is asked to look at a black spot marking the position where the bar of dim light will later appear when the bright light goes out. During this period of thirty seconds, the name, date, etc., can be recorded on a card and the voltage checked. As the watch hand crosses thirty seconds the bright light is turned off and the subject reminded to say "Up" or "Across," indicating the direction of the rectangle seen, as soon as the direction can be made out. The time is noted when this is said, and represents the recovery time from thirty seconds of standard bright light.

"Tests are repeated at minute intervals, three usually sufficing. In some cases the three values may be rather divergent, especially when the first value is long. It is then considered more accurate to use the median or midvalue, rather than the mean; e.g., of three values 23, 15, 16, use 16 rather than 18. The range of recovery times is from three to sixty seconds, with a mode at eight. Thirty to forty persons an hour have been tested by one operator with one instrument.

"*Discussion of Apparatus*—Previous investigations in this field have been hampered by instruments too slow and complicated to permit the testing and retesting of large numbers of people, thereby lacking statistical weight for their findings. Hence the insistence on a test that would be rapid and simple. Every step in the development of this design was tested on numerous subjects not for the accuracy with which it would show new laws of optic physiology, but rather for the avoidance of complications in practice when testing for vitamin A deficiency. Repeated tests were made on each person at each stage, permitting calculations of means, and their standard errors, thus giving mathematical assurance of the validity of every modification. The result is a nice balance, obtained by experimentation of numerous factors, including the colour, duration and intensity of the bright light, the size, colour, duration, and intensity of the dim light, and the area and location of the retina affected and other points.

"Reflection of the bright light from paper makes it more diffuse than reflection from or transmission through glass. Diffusion of the light helps to avoid the afterglow in the eyes from bright spots of light that causes some confusion otherwise. Confusion may also be lessened by having the outer edges of the reflecting surface actually, but not apparently, brighter than the center where the dim light is to be seen. This is done by having a circle of lights for bright illumination.

"Having the dim light in the form of a small rectangle which may be vertical or horizontal gives a more constant recovery time and eliminates guessing

which is common in children. A suitable size was found that would be independent of astigmatism in the subject. Glasses are worn, if they are usually worn, only for the sake of speed, since no effect of removing them has been found, even in extreme myopia and hyperopia. There must, of course, be light transmission and perception. The terms "Up" and "Across" are used because they are much more familiar to the average subject and much faster than any others tried.

"Since the standard error of the mean has been found to be about one second for many people, checks are required only within about one second, and the "reaction times" of subject and operator are unimportant. A practice effect—tests gradually getting shorter— has sometimes been observed if more than three tests were made at one-minute intervals, but if the interval was lengthened to five or ten minutes this effect disappeared."

Results

1. Effects of Antecedent Light

Thomson *et al.* (8, 9) have criticized all visual tests of vitamin A deficiency in regard to inadequate consideration of the effects of previous exposure to light. They consider that the eyes must be fully dark adapted; this requires at least 15 min. in the dark. They report a pronounced effect on their tests if this procedure is not carried out. Since their tests required another 15 or 20 min. to perform, it was not usual to do several tests on one individual. The design of the Pett apparatus permits several tests in a short space of time (three tests in five minutes), giving some mathematical reliability to the results. This procedure is not only easier on patient and operator, but obviates the necessity of trying to achieve the somewhat illusory 'perfect' dark adaptation.

Tables I and II show that this approach overcomes any difficulty from antecedent light. Although the first of the prescribed three tests may be longer than normal (in about one-third of the cases) the subsequent tests are usually uniform. It is not suggested that the eyes after the first test are in

TABLE I

THE EFFECTS OF PREVIOUS EXPOSURE TO LIGHT ON THE TEST FOR VITAMIN A DEFICIENCY

Figures give recovery times in seconds. Three tests were done at one minute intervals, and the median is the value used

Subject	Ordinary tests		Tests after looking at a 1000 watt lamp for:							
			2 min.		5 min.		15 min.		30 min.	
		Med.		Med.		Med.		Med.		Med.
G.A.L.	8, 6, 6	6	8, 6, 5	6	–	–	9, 6, 5	6	–	–
L.B.P.	6, 6	6	7, 6, 7	7	–	–	7, 7, 6	7	–	–
F.H.I.	11, 7, 6	7	–	–	5, 6, 7	6	–	–	9, 7, 6	7
J.A.N.	7, 9, 10	9	–	–	13, 6, 7	7	–	–	–	–
R.C.	4, 6, 6	6	–	–	–	–	–	–	8, 6, 6	6
P.B.	11, 9, 9	9	12, 10, 9	10	–	–	13, 9, 9	9	–	–

TABLE II

THE EFFECTS ON THE TEST OF VARIOUS AMOUNTS OF SUNLIGHT

The median of three tests, expressed in seconds, is used

Subject	Ordinary tests		Tests after exposure to:					
			Bright sunlight		Bright sun on snow		Late afternoon sun, about 1000 ft.-c.	
		Med.		Med.		Med.		Med.
L.B.P.	9, 8, 8	8	9, 8, 10	9	21, 8, 8	8	8, 7, 7	7
D.S	12, 14, 13	13	13, 14, 15	14	18, 15, 15	15	–	–
M.L.	13, 11, 10	11	19, 11, 8	11	–	–	–	–

a physiologically constant condition (if such can ever be), but they are in a sufficiently standard condition to give reproducible results, correlated with vitamin A (7). It will be seen in the tables that the median for a given subject remains constant, within the accuracy expected of the method, regardless of the previous exposure to light.

2. *Effects of Wearing Glasses*

Subjects who enter the test room wearing glasses are told to leave them on, unless they are tinted, since this saves time and avoids the danger of breaking them by leaving them exposed. Most people who wear glasses all the time require a moment's adjustment after removing them. Following this period the Pett test has been found to be the same as before removing the glasses.

TABLE III

THE RECOVERY TIMES OF PERSONS BEFORE AND AFTER REMOVING GLASSES

Median of three tests is given in seconds

	T.R.	R.H.	B.P.	H.W.	A.N.	S.L.	E.L.	J.Mc.	L.F.	A.L.	J.C.	E.P.	J.G.
On	5	8	9	10	10	10	11	11	12	13	17	21	27
Off	6	7	8	10	11	9	10	12	10	11	19	18	27

The results in Table III show clearly that leaving the glasses on does not affect the test seriously. More than 50 persons have been tested in this way, and the conclusion may be drawn that this test is not appreciably affected by myopia, hyperopia, or astigmatism.

3. *Effects of Contracting the Pupils*

Feldman (1) has advocated the use of pilocarpine before a test. Table IV shows our results using this procedure.

In this experiment the subjects had some distress in becoming accustomed to their condition. Although this feeling wore off before the effects of the

drug wore off, it is clear from the table that a great prolongation of the recovery time was caused. In addition great variation in the results was observed. The procedure is therefore considered unsatisfactory.

TABLE IV

RECOVERY TIMES OF SIX PERSONS BEFORE, AND ONE OR TWO HOURS AFTER, THE ADMINISTRATION OF TWO DROPS OF 1% PILOCARPINE NITRATE

Subject	Normal test	Test 1 hour after	Test 2 hours after
A.L.	12	45	16
W.D.	12	57	24
M.P.	6	18	11
B.P.	9	77	32
P.B.	16	80	33
M.L.	9	24	18

4. *Lightness of the Test Room*

The possibility that extraneous light would bother the subject and affect the results of the test has been suggested by all investigators, but no systematic study has been reported. The custom is to use a completely dark room, but in practice this is not always convenient. The Pett apparatus (6) introduced one advantageous procedure by incorporating the bright light within itself, so that the subject's head is in the head-piece all the time.

Stress was also laid on the use of an area of bright light large enough to affect a suitable area of retina, and on the need for uniformity of light over this area. These points have since been emphasized by Thomson (8, 9).

TABLE V

THE RECOVERY TIMES (MEDIAN OF THREE TESTS) OF PERSONS TESTED WITH SIX DIFFERENT LIGHT INTENSITIES IN THE TEST ROOM

Subject	Amount of light, outside the head-piece, in foot-candles					
	Dark	1½	2	4	5	6
T.R.	5	7	7	7	6	5
M.P.	6	8	8	8	7	8
A.L.	7	6	11	11	10	11
R.C.	8	11	12	10	9	7
E.L.	8	10	17	8	11	10
J.C.	10	12	10	11	13	14
A.W.	10	7	10	10	8	9
M.H.	10	9	9	12	9	11
M.P.	12	12	12	12	13	12
V.D.	12	10	12	9	9	9
J.J.	15	17	13	12	14	18
M.L.	14	16	13	20	15	15
D.B.	14	15	13	17	10	19
B.F.	20	26	23	24	27	25
M.M.	24	17	22	21	22	21

Table V shows the effects of varying the amount of light in the test room on the recovery times of various persons. The light in the room is expressed in terms of foot-candles at the head-piece of the apparatus, which is placed so that no bright light, direct or reflected, comes from behind the subject.

The table shows that great variation was found from one person to another in the effect of light in the test room, some cases showing a marked lengthening of the test. Nevertheless, some light may safely be admitted into the room. This amount of light is taken as 1½ ft.-c. at the head-piece, and corresponds to the light from a 200 w. frosted lamp at a distance of about 15 feet. Table lamps can be used in suitable positions in the room, without materially altering this value. Since some people are bothered, even by this much light, routine testing should be carried out in a corner as obscure as convenient.

5. *Effects of Time of Exposure to the Bright Light*

Different workers use different intensities of bright light (not always clearly stated) and also different times of exposure to the light. A great deal of work was done in this connection in evolving the Pett apparatus, but only one interesting point will be mentioned here.

Twenty persons were used in an experiment in which three tests were given at each of 10 different exposure times, varying from 5 to 60 sec. Typical curves for three persons are given in Fig. 1.

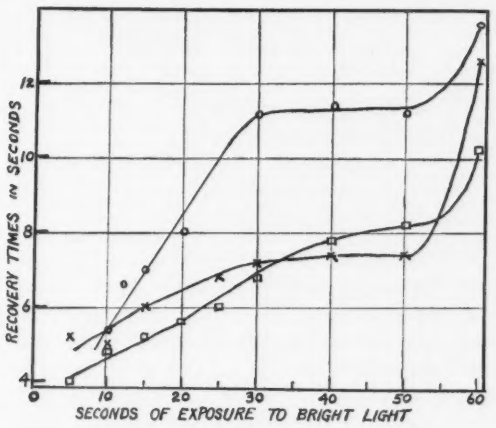

FIG. 1.

It will be seen in Fig. 1 that each curve has a flattened part (or constant Recovery Time) for a range of about 30 to 50 sec. exposure. The flat part of the curve varies slightly with each individual, and at different times with the same individual. A similar result was found when the time was held constant and the brightness of the light varied. Within the range of this flat part easily reproducible measurements can be made, and the recovery

times of all 20 people studied could be safely measured with the 30 sec. exposure prescribed for the test. Some evidence was found that the curve again flattens out with much brighter lights or longer exposure times. Restlessness and fatigue of the subjects are more marked when looking at the bright light more than one minute, or at very bright lights, so these are not considered suitable conditions for this test.

6. Practice Effects

The important possibility of people becoming so acquainted with the test that the results show improvement without dietary cause has received only slight attention. In actual practice, where it is desired to follow the course of treatment by repeated tests, such a 'learning' effect might lead to erroneous conclusions. With rapid tests, repeated testing is encouraged, and consequently extensive investigation of the possibility of "learning" has been carried out with the Pett test.

Careful examination by one of the writers (L. B. P.) of the last three years' results, during which nearly 4,000 persons have been tested, did not show much suggestion of practice effects. More than 500 persons have had many repeated tests without showing a "learning" effect. In addition some experiments have been designed especially to elucidate this point.

A group of 17 persons received 15 tests each, with only one minute interval between tests. One person showed a significant practice effect, i.e., the first few recovery times gradually became shorter until a significantly lower level was reached. Lengthening the interval between tests to two minutes removed this effect.

A different group of 25 persons, never before tested, received a single test daily (not the prescribed three tests) for 21 days. Of the 25, 11 persons showed learning curves. Thus with one observation only learning may be a disturbing factor in certain cases, but the procedure prescribed for this test, requiring three tests, successfully guards against this error.

7. Dispersion or Variability of Tests on One Person

The arithmetical mean (or median) of two or more figures represents those figures with a degree of accuracy depending on how much the figures differ from each other, and from the mean or median. The usual measure of such dispersion is the standard deviation:

$$\sigma = \sqrt{\frac{\Sigma\,(d^2)}{n-1}}.$$

This deviation marks off a distance above and below the mean or median, making its accuracy comparable with other similarly calculated means. The standard error of the mean (or median) expresses the limits within which will fall any future mean (or median) of the same experiment, no matter how many times the experiment is repeated. It is the result of dividing the standard deviation of the observations by the square root of the number of observations.

The latter calculation was carried out on every twentieth card in the writers' files, until 100 had been done. Table VI summarizes some statistical information resulting. It will be seen that the most likely deviation is 0.9 if the mean is used, and 1.7 if the median is used. The use of the median was recommended by Pett (6) owing to convenience. Actually in more than half the cases it gives just as great accuracy as using the mean, even though the above figures show a greater error. This means that observations within two seconds of each other are satisfactory. It may be noted that with recovery times of more than 20 sec. the variation is greater, but the percentage error remains about the same.

TABLE VI

STANDARD ERRORS OF THE MEAN (OR MEDIAN) OF THE PRESCRIBED THREE TESTS ON EACH OF 100 PERSONS, TAKEN AT RANDOM FROM 2,000 CASES

The figures are in seconds

Of three tests	Range of deviations		Mean of deviations	Mode of deviations
	Low	High		
Means	±0	±4.4	±1.4	±0.9
Medians	±0	±8.9	±2.4	±1.7

8. *Possibility of a Diurnal Rhythm*

Since vitamin A storage is most marked in the liver, some functional role of this organ in vitamin A metabolism might be suspected. The liver is believed by some (4) to change in activity at different times of day, in releasing bile and sugar, so it seemed possible that vitamin A might be similarly affected. If changes during the day in the vitamin A content of the blood, reflected in a visual test, were large, they might be an important factor in deciding when such visual tests should be done. Since this test has been correlated with blood analysis (7) it may be taken as an indication of the blood level. Tests were therefore done at intervals during the day. These are summarized in Table VII. Table VII shows that in non-fasting persons the tests varied from one time of day to another. Great individual variation was found, but a tendency may be noted to show low values in the early morning, increasing to a peak late in the morning, and decreasing again. These data are suggestive but not conclusive. The idea of a change during the day receives support from the results on a fasting person (L. B. P.) also shown in Table VII. In this case the values were low in the early morning (indicating high blood vitamin A), high at 11 a.m., decreased to 7 p.m., increased and later decreased again. These figures are averages of five tests at each time. The standard error of the differences between any two figures in this fasting experiment is ±0.25, so most of the differences are significant. (i.e., more than twice the standard error). These results are not subject to the influences of current food intake or exercise, but they cannot be taken

as conclusive until more tests are made. There is, however, a definite suggestion of a diurnal rhythm of vitamin A in the blood.

TABLE VII

RECOVERY TIMES (IN SECONDS) AT INTERVALS DURING THE DAY OF *(a)* A FASTING PERSON, AND *(b)* SEVERAL PERSONS NOT FASTING

Longer times mean lower vitamin A

Time of day	Fasting	Not fasting						
		1	2	3	4	5	6	7
8 a.m.	5.8	–	–	–	–	–	–	–
9 a.m.	5.6	20	8	7	12	4	14	10
10 a.m.	7.4	24	12	7	16	5	11	13
11 a.m.	7.5	34	12	10	10	6	14	14
12 noon	7.2	24	11	14	11	6	13	15
1 p.m.	6.8	–	–	–	–	–	–	–
2 p.m.	7.6	29	9	8	11	6	13	13
3 p.m.	7.2	29	9	12	13	5	13	11
4 p.m.	6.7	25	7	15	12	6	16	13
5 p.m.	6.8	25	6	11	8	4	12	12
6 p.m.	6.5							
7 p.m.	6.3							
8 p.m.	7.1							
9 p.m.	7.2							
11 p.m.	6.5							
8 a.m.	6.0							

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THE COMPOSITION OF THE "5 : 3" CALCIUM ALUMINATE[1]

By T. Thorvaldson[2] and W. G. Schneider[3]

Abstract

When the isotropic hexahydrate of tricalcium aluminate is dehydrated, calcium oxide is liberated with the formation of a calcium aluminate lower in lime. A quantitative study of this reaction was made in order to determine the composition of the aluminate formed. Errors due to impurities in the tricalcium aluminate used, incompleteness of hydration to the hexahydrate, carbonation during treatment, retention of water by the final product, and recombination of the free lime with the aluminate formed were eliminated. The results indicated the formation of an aluminate of the composition $12CaO \cdot 7Al_2O_3$. The aluminate formed during the dehydration was found to give the same X-ray diffraction pattern as apparently homogeneous samples of the compositions $5CaO \cdot 3Al_2O_3$ and $12CaO \cdot 7Al_2O_3$ prepared directly from lime and alumina by thermal methods. The results support the assumption that the so-called "5 : 3" calcium aluminate should be assigned the formula $12CaO \cdot 7Al_2O_3$.

In their pioneer investigation on the system CaO–Al_2O_3 Shepherd, Rankin, and Wright (6) prepared four aluminates of calcium to which they assigned the compositions $3CaO \cdot Al_2O_3$, $5CaO \cdot 3Al_2O_3$, $CaO \cdot Al_2O_3$ and $3CaO \cdot 5Al_2O_3$. In 1931 Koyanagi (4), after studying melts of lime and alumina in various ratios, suggested the existence of an aluminate of the composition $3CaO \cdot 2Al_2O_3$. Later, on the basis of structural analysis, Büssem and Eitel (2) proposed the formula $12CaO \cdot 7Al_2O_3$ instead of the 5 : 3 ratio. A study of X-ray patterns of crystallized melts led Lagerquist, Wallmark, and Westgren (5) to a similar conclusion, although they considered the composition $9CaO \cdot 5Al_2O_3$ as an alternative possibility.

It is difficult to obtain conclusive results in the case of compositions as close together as the 5 : 3 and 12 : 7 lime–alumina mixtures (47.8 and 48.5% CaO) by either the microscopic study of quenched melts or by X-ray diffraction studies. It would, therefore, be desirable to find other experimental methods which would give evidence as to the true composition of the aluminate.

Shepherd, Rankin, and Wright (6) found that tricalcium aluminate melts incongruently producing free lime and the next aluminate lower in lime. If this decomposition could be made quantitative and the free lime in the product determined, the composition of the aluminate formed could be calculated. It is, however, unlikely that this could be done with the necessary accuracy. Thorvaldson and Grace (7) found that the hexahydrate of tricalcium aluminate decomposes in a similar manner when dehydrated at temperatures above 275° C. The maximum liberation of lime observed was 26.6% (anhydrous basis) while the formation of the 5 : 3 aluminate calls for liberation of 27.7% free lime. Büssem (1, p. 150) cites this result as evidence for the correctness

[1] *Manuscript received February 8, 1941.*
Contribution from the Department of Chemistry, University of Saskatchewan, Saskatoon, Sask.

[2] *Professor of Chemistry.*
[3] *Holder of a Bursary under the National Research Council of Canada.*

of the formula $12CaO \cdot 7Al_2O_3$, which would require 26.69% free lime. There are, however, several factors that may interfere with the accuracy of the determination of the maximum free lime liberated on decomposition of the hexahydrate of tricalcium aluminate, and the experimental work of Thorvaldson and Grace (7) was not done with the object of determining the exact composition of the anhydrous aluminate formed. It was therefore decided to make a careful quantitative study of the thermal decomposition of $3CaO \cdot Al_2O_3 \cdot 6H_2O$.

It is evident that the tricalcium aluminate used must be very pure, with no appreciable excess of either lime or alumina, and that it must be converted completely to the hexahydrate. Incomplete hydration would result in incomplete thermal decomposition of the aluminate at the temperatures used, and would therefore give low values for the free lime liberated. In the lower part of the range of temperatures causing decomposition of the hexahydrate with liberation of lime, the dehydration is never complete. If this retention of water is due to the presence of some undecomposed hexahydrate, the results for free lime in the product would be low. However, if temperatures that are high enough to cause complete dehydration are used there is always some recombination of the lime and the lower aluminate to give anhydrous tricalcium aluminate, again resulting in a low maximum value for the free lime. Any contamination with carbon dioxide of the air during the dehydration would also give low results. It will thus be seen that all the most likely errors would give low results for the free lime in the decomposition mixture and thus lead one to favour the formula $12CaO \cdot 7Al_2O_3$ as against the formula $5CaO \cdot 3Al_2O_3$ for the lower aluminate. The object of the present investigation was to eliminate every possibility of error in the determination of the maximum free lime liberated on the decomposition of the hexahydrate of tricalcium aluminate and thus to obtain reliable evidence as to the composition of the aluminate formed. Table I shows the maximum amount of free lime to be expected according to each of the formulae mentioned above.

TABLE I

CALCULATED LIME MAXIMA FOR THERMAL DECOMPOSITION OF $3CaO \cdot Al_2O_3 \cdot 6H_2O$

Composition of final product	Maximum percentage free lime expected
$3CaO \cdot 2Al_2O_3$	31.13
$5CaO \cdot 3Al_2O_3$	27.67
$12CaO \cdot 7Al_2O_3$	26.69
$9CaO \cdot 5Al_2O_3$	24.91

Experimental

Preparation of $3CaO \cdot Al_2O_3 \cdot 6H_2O$

Calcium carbonate (low alkali analytical reagent) was dissolved in freshly redistilled hydrochloric acid and the solution made alkaline by addition of calcium oxide, prepared from the same material, to precipitate traces of iron,

alumina, etc. After filtering, the solution was diluted and the calcium precipitated by means of pure ammonium carbonate. The ammonium carbonate contained no insoluble matter and negligible non-volatile residue. The calcium carbonate was then washed free of chloride, dried, and analyzed. The analysis gave 56.10% CaO.

The alumina was prepared from "iron-free" ammonia alum by double precipitation using freshly redistilled ammonia and hydrochloric acid and washing free of sulphates and chlorides. The alumina after drying at 105° C. was found to contain 69.15% Al_2O_3 and less than 0.01% Fe_2O_3.

The calcium carbonate and alumina were then weighed out in the required proportions, mixed in a stoppered bottle, transferred to platinum crucibles, and the carbon dioxide and most of the water slowly driven off in a muffle furnace (maximum temperature 850° C.). The platinum crucibles were then heated in an induction furnace at 1450 to 1500° C. for 20- to 50-min. periods with intermediate grinding in an agate mortar to pass a 200 mesh sieve. Samples *A* and *B* contained 2 and 8% free lime respectively after the first heating in the induction furnace, while the last trace of free lime disappeared after the seventh and the fifth heatings, respectively. Examination of the product by means of the petrographic microscope showed it to be homogeneous and isotropic with a refractive index of 1.710. The analysis is given in Table II.

TABLE II

ANALYSIS OF IGNITED SAMPLES OF TRICALCIUM ALUMINATE

	Experimental		Calculated
	A	*B*	
Per cent CaO	62.24	62.27	62.27
Per cent Al_2O_3	37.80	37.86	37.73

Small samples of the aluminate were hydrated in platinum crucibles in an autoclave, which was heated slowly to 150° C. and kept at that temperature completely protected from contamination with carbon dioxide. In some cases the platinum crucibles containing the samples were placed over water in small silver lined steel autoclaves, which were heated in an electric oven at 120° C. After hydration, the samples were dried *in vacuo* over freshly ignited lime. All the samples were examined by means of the polarizing microscope and only those that were homogeneous and completely isotropic were used for the dehydration experiments. Eleven separately prepared samples were used for the final series of dehydrations. The hydration period varied from 5 to 14 days, the water content from 39.93 to 40.09% (which corresponds to a molar ratio of 5.98 to 6.01). The refractive index of the hydrate was 1.604.

The Thermal Decomposition of $3CaO \cdot Al_2O_3 \cdot 6H_2O$ *and the Determination of Free Lime*

The furnace was a vertical fused silica tube, 24 by 1 in., the middle portion being wound with a heating element and insulated with alundum and asbestos packing. The temperature was measured by means of a calibrated platinum–platinrhodium thermocouple, the junction being placed just above the 4 cc. platinum crucible containing the hydrate. To avoid violent evolution of moisture the sample was placed in the cold furnace and the temperature gradually raised to that desired. A slow current of air, thoroughly purified and then dried by passing over soda-lime and finally over anhydrous magnesium perchlorate, was passed upwards through the furnace during the heating. On removal, the sample was cooled over freshly ignited lime in a desiccator and the crucible after weighing transferred directly into a boiling-flask containing 60 cc. of 1 : 5 anhydrous-glycerol–alcohol mixture. The free lime was then determined according to the method of Lerch and Bogue. The end-point was very definite, except with the samples dehydrated at low temperatures (275 to 500° C.), and most of the lime was titrated within the first ten minutes while the end-point was usually reached within one hour. Using 0.5 gm. samples containing about 26% free lime, replicate determinations usually agreed within $\pm 0.05\%$. Contamination with carbon dioxide, which is probably the most serious cause of error, was carefully avoided.

The first indication of the decomposition of the aluminate appears on prolonged heating of the hexahydrate at about 275° C., when the dehydration product turns pink when boiled in the glycerine-alcohol-phenolphthalein mixture. Higher temperatures must, however, be used before any free lime is readily extracted, and the free lime determination is somewhat uncertain when the dehydration temperature used is below 500° C.

In all cases the product of dehydration contained considerable amounts of water, as shown by ignition at 1100° C. to constant weight. A series of dehydrations at various temperatures was therefore made to ascertain whether the amount of water retained affects the amount of lime liberated. A short period of heating (three hours) was chosen to reduce the amount of possible recombination of lime with the aluminate. The results are given in Table III.

TABLE III

COMPARISON OF WATER RETAINED AND LIME LIBERATED

Temperature, °C.	Time of heating, hr.	Per cent water retained	Per cent CaO liberated
550	3	1.92	26.63
650	3	1.49	26.66
750	3	1.38	26.64
850	3	0.77	26.58
950	3	0.74	26.61

It is evident from the data of Table III that the amount of lime liberated remains constant over a considerable range of temperature while the amount of water held varies, and, that, when the water content of the product drops below approximately 2%, further dehydration does not affect appreciably the amount of lime liberated. This indicates that in a three-hour heating in a very dry atmosphere at 550° C. or above, none of the hexahydrate of tricalcium aluminate remains undecomposed. Possible error from this source is thus eliminated.

Three series of determinations of the lime liberated on heating the isotropic hexahydrate for various periods of time at 650, 750, and 850° C. were also made for the purpose of determining definitely the rate of recombination of the lime and the aluminate formed. The platinum crucibles containing the hexahydrate were placed in the cold furnace to prevent the sudden liberation of water with possible hydrolysis, and a fresh sample was used for each run. The results are given in Table IV and in Fig. 1.

TABLE IV

THERMAL DECOMPOSITION OF TRICALCIUM ALUMINATE HEXAHYDRATE

Temperature, °C.	Time of heating, hr.	Per cent water retained (anhydrous basis)	Per cent free CaO (anhydrous basis)
650	1	6.09	23.58
	2	1.84	26.33
	3	1.60	26.60
	6	1.36	26.63
	12	1.49	26.62
	24	1.35	26.50
750	1	0.86	26.64
	3	1.05	26.66
	6	1.03	26.63
	12	0.80	26.54
	24	0.72	26.24
850	1	0.81	26.65
	3	0.61	26.60
	6	0.60	26.44
	12	0.85	26.29
	24	0.63	26.03

From Table IV it is apparent that at 650° C. the decomposition is not complete until the heating has continued from three to six hours and that the rate of recombination is approximately 0.1% of lime in 12 hr. At 750° C. the decomposition appears complete in from one to three hours, with recombination apparent between 6 and 12 hr. at a rate of approximately 0.3% of lime in 12 hr., while at 850° the decomposition is probably complete in one hour and the average rate of recombination is somewhat greater than at 750°. The value for the free lime obtained by extrapolation to zero time

(Fig. 1) in order to eliminate the effect of recombination does not differ appreciably from the maximum for each curve.

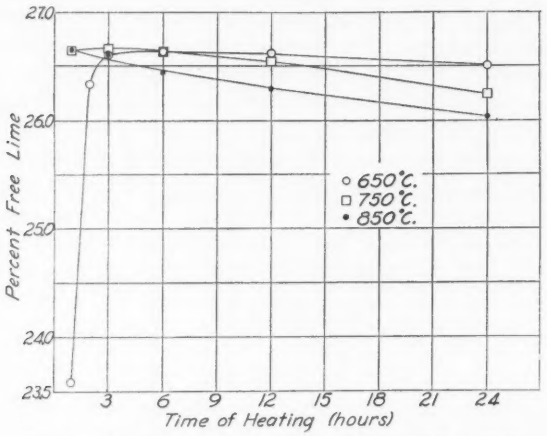

FIG. 1. *The thermal decomposition of* $3CaO \cdot Al_2O_3 \cdot 6H_2O$.

Considering the results as a whole, there does not seem to be any reason to suspect that the value obtained for free lime might be too high. It would therefore appear that the possibility of the composition $9CaO \cdot Al_2O_3$ (corresponding to 24.91% free CaO) may be eliminated. The formula $3CaO \cdot 2Al_2O_3$ (31.13% free CaO) may also doubtlessly be ruled out. The experimental evidence supports strongly the composition represented by $12CaO \cdot 7Al_2O_3$ rather than $5CaO \cdot 3Al_2O_3$ as the aluminate formed on dehydration.

There remains the question whether the anhydrous aluminate obtained after dissolving the free lime from the mixture obtained on dehydration of the hexahydrate of tricalcium aluminate is identical with the aluminate obtained by thermal treatment of the corresponding mixture of lime and alumina. X-ray powder patterns were made using the solid residue remaining after the determination of free lime and the apparently homogeneous products obtained on thermal treatment of lime and alumina in the ratios $12CaO \cdot 7Al_2O_3$ and $5CaO \cdot 3Al_2O_3$. All the X-ray patterns appeared identical and the readings were those given by Harrington (3) for the product $5CaO \cdot 3Al_2O_3$.

It therefore appears that on dehydration of the hexahydrate of tricalcium aluminate a crystalline aluminate of the composition $12CaO \cdot 7Al_2O_3$ (48.53% CaO and 51.47% Al_2O_3) is formed with the liberation of 26.7% of calcium oxide, and that X-ray diffraction photographs show that the same product is obtained by direct thermal combination of lime and alumina in the above proportions. If the formula $5CaO \cdot 3Al_2O_3$ were to be accepted as representing the composition of the compound, it would be necessary to assume that in all

the experiments described above, 98.66 parts of this compound retain 1.34 parts of calcium oxide in solid solution, and that this dissolved lime cannot be extracted under the conditions existing during the determination of free lime. Without further evidence such an assumption appears unwarranted.

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PASTURE STUDIES. XIX.

A SIMPLIFIED APPARATUS FOR THE CONTINUOUS EXTRACTION OF MOISTURE AND FAT FROM BIOLOGICAL MATERIALS[1]

By E. W. Crampton[2] and T. L. Purdy[3]

Abstract

A modification of the Kaye apparatus for the determination of moisture by distillation and of lipids by isopropyl ether extraction is described. The modified apparatus has been used in the analysis of plant material and faeces, and typical results are presented. It appears that isopropyl ether and ethyl ether yield similar amounts of extract, but that oven-drying may result in values that are too low.

The present chemical methods of analysis of food stuffs and of faeces are being re-examined from a biological standpoint and one fraction under consideration is the ether extract. Two criticisms of the standard A.O.A.C. method have been raised. One is that the process of oven-drying, to which samples are subjected, is liable to volatilize certain fractions of the ether extract and also theoretically, at least, may permit oxidation of the extractives. In the second place, the 'ether extract' includes a variety of substances of differing nutritional significance depending on the parent material.

A method of analysis that would eliminate the necessity of drying the sample by heat should give a truer picture of the composition of the lipid fraction. Kaye (1) has proposed such a procedure and has designed an apparatus that in principle consists of a water collection trap combined with a Soxhlet type extractor. The method requires a fat solvent, insoluble in water, and with a boiling point close to that of water. Isopropyl ether possesses the desired properties. With it, Kaye extracted from human faeces 30% more neutral fats than was obtained by standard methods with ethyl ether.

This procedure seemed to be applicable to the solution of one of the problems involved in the study of the nutritive value of pasture herbage. The design of the apparatus, however, appeared more complicated and expensive than necessary, and an attempt was made in this laboratory to simplify it, particularly with respect to: (i) quantities of solvent required per unit of sample, (ii) ease of assembly, (iii) operation as to temperature control, and (iv) reduction in the number of separate parts and standard joints necessary.

The new design (Fig. 1) is based on the standard Soxhlet apparatus and includes the following modifications of the Kaye apparatus:

[1] *Manuscript received January 20, 1941.*

Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que. Journal Series No. 155.

[2] *Associate Professor of Animal Nutrition.*

[3] *Research Associate, Nutrition Section, Department of Animal Husbandry.*

1. The ground glass joint between the boiling-flask and the extraction chamber was eliminated. This was thought advisable in that the entire unit is immersed in a water or steam bath.

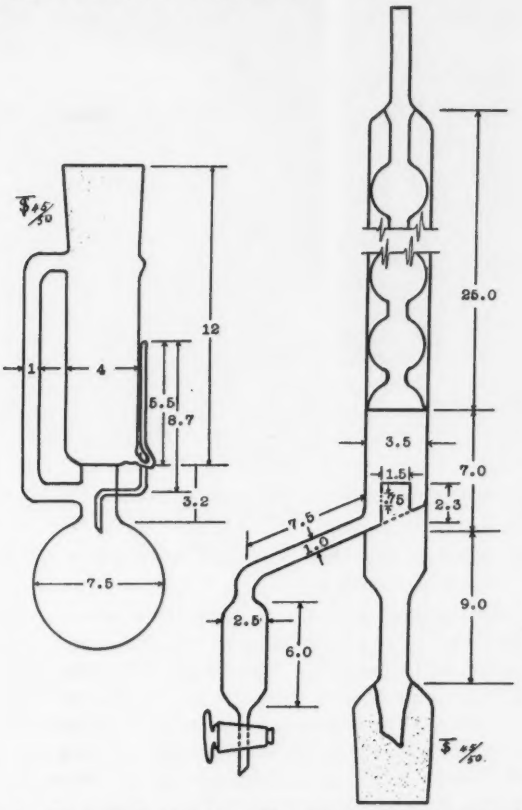

FIG. 1. *Diagram of apparatus. All measurements are in centimetres.*

2. Attached to the condenser and above the standard ground glass joint between the condenser and the extraction chamber is a water-solvent separator, connected by an offset tube to a water trap. The essential parts of this are: *(a)* a sloping floor leading to an offset collection bulb, fitted with a stopcock so that the water collected may be drained off completely and measured or weighed; *(b)* a central tube in this floor through which the ascending vapours may pass without interference from the returning condensate. The condensate runs down the walls of the collection chamber, and the ether returns through an aperture in the side of the vapour conduction tube. In this way the droplets of water are not so readily carried back to the thimble by surface

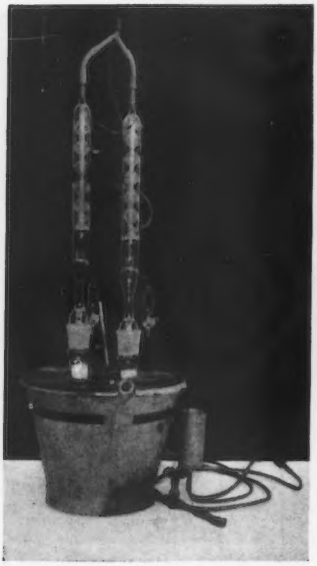

FIG. 2. *Apparatus assembled in deep steam bath.*

FIG. 3. *Inside view of steam bath showing arrangement of steam coil and rubber seats for extractors.*

tension of the solvent, since most of them must pass around the circumference of the collection chamber.

Heating is accomplished by means of a deep steam bath. This was made from a pail that was of a depth sufficient to enclose the extracting unit to the level of the top of the thimble. Three turns of flexible metal tubing about the lower inside circumference of the bath, attached to a steam line, supplies the heating element. The water is kept at constant level by means of a constant level chamber on one side of the bath. The water is maintained at a level sufficient to cover the flasks of the unit as it stands on a notched rubber ring on the bottom of the bath. The weight of the apparatus overcomes the buoyancy of water of this depth and with the rubber ring seat, clamps other than for the condenser are unnecessary. One such steam bath accommodates two extractor units. A divided cover, cut out to accommodate the units, completely encloses the extraction units. A rubber mat cut to fit exactly around the extraction chambers prevents escape of steam. Thus the whole extraction unit may be heated uniformly at a constant temperature, regulated by the steam allowed into the coils.

Operation

Two steps are involved in the procedure.

(I) A standard thimble 33 by 80 mm. is used to hold the sample of approximately 10 gm. As soon as this is placed in the extraction chamber the con-

denser unit is fitted. (A very light coating of glycerol and glucose (1 : 1) was found advantageous on the ground glass joint, making it possible to dismantle the apparatus without waiting for it to cool.) The isopropyl ether is then added through the top of the condenser filling the water collection bulb and the separation chamber to the aperture in the vapour conduction tube.

Additional ether is added to cover the sample in the thimble but not more than sufficient to rise half-way up the syphon. From 65 to 75 ml. are needed for the water distillation, the amount depending on the bulk of the sample. If more than this is added there is, on heating, a tendency for the ether to bubble over the syphon, with the possibility of carrying water soluble material into the flask at the beginning of the distillation. It seems difficult to prevent this in the Kaye apparatus. The use of the deep steam bath with the modified apparatus, completely enclosing the extraction unit, overcomes this difficulty.

Distillation is carried out with the steam bath brought slowly to 85° C. At this temperature no droplets of moisture collect high enough in the condenser to make their removal difficult. Distillation takes from two to four hours, the time depending on the nature of the sample. With material that might become compact, operations can be facilitated by mixing pre-dried asbestos with the sample.

(II) When the distillation is completed, one-half of the cover of the steam bath is raised. This reduces the temperature of the extraction chamber below the boiling point of the ether. Extraction is then continued with the addition of sufficient ether (about 20 to 30 cc.) to allow the syphon to operate as a standard Soxhlet.

The collected water may be removed and measured or weighed. In this laboratory, graduated 15 ml. centrifuge tubes were found useful for this purpose. A small amount of ether drawn off with the water provides an easily read meniscus.

Following removal of the water, the water trap becomes a unit through which the solvent may be recovered merely by closing the cover of the bath and heating the whole unit as for distillation of the water. (Recovery of solvent has been 80 to 85%.) The ether thus recovered requires no further distillation for immediate subsequent use.

On completion of the extraction and recovery of the solvent, the ether extract may be transferred with warm ether to a tared beaker, dried *in vacuo*, and weighed.

Discussion

It is important that freshly prepared, dry ether be used. The writers' results indicate that ether that has been unused for as long as two days should be redistilled before further use. A comparison of the results of extraction with peroxide-free and peroxide-containing ether emphasizes this point (Table I). Not only was there a difference in weight of extract obtained from fresh cereal grass, but also the green colour of the extract obtained with peroxide-free ether was completely destroyed by the peroxide-containing

solvent. Determinations also indicated a complete loss of carotene in extracts obtained with peroxide-containing ether.

TABLE I

RESULTS OF EXTRACTION OF CEREAL GRASS IN MODIFIED APPARATUS, SHOWING THE EFFECTS OF PEROXIDE IN THE SOLVENT

	Extracted with isopropyl ether, peroxide-containing	Extracted with isopropyl ether, peroxide-free
Original sample	100	100
Water	74.0	73.2
Ether extractives	1.4	1.2
Residue	24.7	24.5
Total accounted for	100.1	98.9
Error	+0.1	−1.1
Per cent loss of extractives on subsequent oven drying	14.6	9.0
Colour of extract	Brown	Green
Carotene in extract	Absent	Normal

It would appear that isopropyl ether extracts do not differ quantitatively from ethyl ether extracts (Table II). Removal of water by distillation, followed by extraction with ether, yields a definitely larger proportion of ether extract from plant materials than does the A.O.A.C. method.

TABLE II

COMPARISONS OF METHODS OF MOISTURE AND LIPID DETERMINATION ON FRESH FAECES, FRESH GRASS, AND DRIED GRASS

Material	Fractions	Oven drying at 105° C. followed by Soxhlet extraction		Vacuum drying at 90° C. followed by Soxhlet extraction	Moisture by distillation, followed by extraction with isopropyl ether	
		Ethyl ether	Isopropyl ether	Ethyl ether	Crampton apparatus	Kaye apparatus
Fresh steer faeces	Sample	100.00		100.00	100.00	
	Moisture	82.38		81.66	82.39	
	Ether extract	1.27		1.36	1.22	
	Residue	16.40		15.60	17.40	
	Total accounted for	100.05		98.62	101.01	
Fresh grass	Sample	100.00	100.0		100.00	
	Moisture	79.81	79.49		79.50	
	Ether extract	0.54	0.56		0.62	
	Residue	19.73	19.86		20.85	
	Total accounted for	100.08	99.91		100.97	
Dried cereal grass (commercial)	Sample	100.00			100.00	100.00
	Moisture	7.43			5.67	5.63
	Ether extract	4.30			5.93	5.86
	Residue	88.27 (dif.)			88.76	88.00
	Total accounted for	100.00			100.36	99.49

The authors are indebted to V. K. Collins and R. A. Chapman, Department of Chemistry, Macdonald College, for assistance with the chemical analyses.

Sufficient work has not been done on faeces to make certain that the example cited is typical: but indications are that the ether extractives lost from fresh herbage material by standard procedures are not present in measurable quantities in the faeces of steers fed on such herbage.

That a part of the ether extractable material may be lost through oven-drying certain substances is indicated in Table III.

TABLE III

LOSS OF ETHER EXTRACT BY HEATING IN OPEN OVEN AT 105° C.

Sample	Per cent moisture by distillation	Per cent ether extract by extraction in Crampton apparatus	Per cent ether extract lost by subsequent heating in oven at 105° C. for 12 hr.
Dried grass (commercial)	5.7	5.9	9.4
Fresh pasture grass	79.9	0.6	10.3
Fresh cereal grass (rye)	73.2	1.2	9.0

Presumably the extract was moisture-free and consequently the loss (Table III, Col. 3) was not water but some fraction of the dry ether extract. It is perhaps significant that a comparable loss was obtained with three forms of similar plant material.

Reference

1. KAYE, I. A., LEIBNER, I. W., and CONNOR, E. B. J. Biol. Chem. 132(1) : 195-207. 1940.

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